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Enhancement of osteogenesis in vitro by a novel osteoblast differentiation-promoting compound, TAK-778, partly through the expression of Msx2

Masayuki Gotoh *, Kohei Notoya, Yuka Ienaga, Masahiro Kawase, Haruhiko Makino

Pharmacology Research Laboratories I, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa, Osaka 532-8686, Japan

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Abstract

TAK-778 [(2*R*,4*S*)-(−)-*N*-(4-Diethoxyphosphorylmethylphenyl)-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepin-2-carboxamide: mw 505.52], a novel compound promoting osteoblast differentiation, promotes osteogenesis in vitro and enhances bone formation during skeletal repair in vivo. In this study, we further evaluated the effects of TAK-778 on the differentiation of cultured bone marrow stromal cells into osteoblasts in the presence of dexamethasone, paying particular attention to the expression of transcription factors involved in regulating osteoblast differentiation. Treatment of TAK-778 (10^{-7}–10^{-5} M) for 4 h resulted in an increase in the mRNA expression of Msx2, but not Cbfa1 or Dlx5. This transcriptional alteration preceded the changes in other markers related to the osteoblast phenotype, such as alkaline phosphatase and osteocalcin mRNA. The transfection of Msx2-antisense in the cells caused a significant reduction in the levels of alkaline phosphatase mRNA expression induced by TAK-778. These results suggest that TAK-778 promotes osteoblast differentiation partly through the expression of Msx2, a homeobox-related gene.

Keywords: Osteoblast differentiation; Alkaline phosphatase; Growth factor; Msx2; TAK-778

1. Introduction

Bone tissues have a remarkable ability to regenerate. Fracture healing is a biologically important process resulting in the restoration of injured skeletal tissue to a state of normal structure and function (Cruess and Dumont, 1985). However, some events, including aging, poor blood supply, and diabetes, may lead to prevent fracture healing (Buckwalter et al., 1996). The restoration of an osteoporotic fracture is also delayed since the mechanical strength of the fracture site is decreased due to an insufficient amount of callus and calcification (Melton, 1995; Walsh et al., 1997). Thus, the development of agents to enhance fracture healing would be a significant pharmacological advancement in terms of not only accelerating healing, but also reducing the risk of delayed- or non-union.

A number of growth factors, cytokines, and their receptors are present in high levels at fracture sites and many of these proteins play important roles in promoting fracture repair (Bolander, 1992). Recent studies suggest that the local application of bone morphogenetic proteins (BMPs) (Nilsson et al., 1986; Yasko et al., 1992), basic fibroblast growth factor (bFGF) (Kawaguchi et al., 1994; Inui et al., 1998), and transforming growth factors (TGF) (Beck et al., 1993) enhances fracture repair in animal models. These findings indicate that the clinical use of these growth factors may make possible new therapies for enhancing fracture healing. However, the safety, utility, and cost-effectiveness of these growth factors must be considered. Therefore, there has been substantial interest in developing a chemical compound that safely promotes bone formation and facilitates fracture repair.

We previously reported that a novel chemical compound, TAK-778 [(2*R*,4*S*)-(−)-*N*-(4-Diethoxyphosphorylmethylphenyl)-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepin-2-carboxamide; mw 505.52] (Fig. 1) enhanced osteogenesis in vitro and in vivo (Notoya et al.,

* Corresponding author. Tel.: +81-6-6300-6871; fax: +81-6-6300-6306.
E-mail address: Goto_Masayuki@takeda.co.jp (M. Gotoh).

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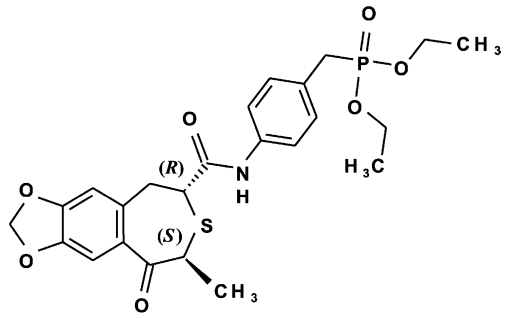

Fig. 1. Chemical structure of TAK-778.

1999). This compound markedly promotes the formation of bone-like nodules in the presence of dexamethasone in rat bone marrow stromal cell culture and increases alkaline phosphatase activity, soluble collagen release, and osteocalcin secretion. Under the culture conditions, TAK-778 also stimulates the secretion of transforming growth factor (TGF)-β and insulin-like growth factor (IGF)-I, indicating that TAK-778 may exert regulatory effects on osteoblast differentiation via an autocrine/paracrine mechanism. Furthermore, local application of TAK-778 using sustained release microcapsules consisting of a biodegradable polymer, copoly (dl-lactic/glycolic acid) (TAK-778/PLGA-MC), enhances bone formation during skeletal regeneration and bone repair in animal bony-defect and fracture models. These results reveal that this non-endogenous compound enhances osteogenesis by promoting osteoblast differentiation and should be useful for the stimulation of fracture healing. However, the mechanisms underlying the stimulatory effect of TAK-778 on osteoblast differentiation remain unclear. In this study, we used quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay to further evaluate the pharmacological profile of TAK-778 on the differentiation of cultured bone marrow stromal cells into osteoblasts in the presence of dexamethasone, with special interest in the transcriptional alteration of growth- and/or differentiation-related proteins. We also investigated the effect of TAK-778 on the expression of Cbfa1, Msx2, and Dlx5, which have been reported to regulate the differentiation pathway of osteoblast (Ducy et al., 2000).

2. Materials and methods

2.1. Materials

TAK-778 was synthesized at Takeda Chemical Industries (Osaka, Japan) by the method of Oda et al. (1999).

2.2. Cell culture

Rat bone marrow stromal cells were prepared and cultured according to the method of Maniatopoulos et al. (1988). Cells were obtained from femoral bone marrow of 7-week-old male Sprague–Dawley rats (Japan Charles River, Tokyo, Japan). The cells selectively differentiated into osteoblast and subsequently formed mineralized bone-like nodules in the presence of β-glycerophosphate and dexamethasone. A standard culture medium consisting of α-minimum essential medium (MEM) containing 10 mM HEPES (pH. 7.0) was used. It contained 15% fetal bovine serum, 2 mM glutamine, 50 μg/ml of ascorbic acid, 10 mM

Table 1
Nucleotide sequences of PCR primers and TaqMan probes

Target		Sequence
ALP	Forward primer	TGGACTACCTCTTAGGTCTCTTTGAGC
	Reverse primer	TCCAGCAAGAAGAAGCCTTTGG
	TaqMan probe	AATCGGAACAACCTGACTGACCCTTCCCT
Osteocalcin	Forward primer	TGAGCTCAACCCCAATTGTGA
	Reverse primer	GCTGGAGAGTAGCCAAAGCTGAA
	TaqMan probe	AAGCGCATCTATGGCACCACCGTTT
Type I collagen	Forward primer	AACACCTATTCCCTCTGATGTACACC
	Reverse primer	GGAACTCAGTAAAGTCCTGCCTTC
	TaqMan probe	TGGGCATCCTTTGGAACTGACCACTG
TGF-β_2	Forward primer	CGGACTACTACGCCAAAGAAGTCAC
	Reverse primer	GCTTCCCGAATGTCTGACGTAT
	TaqMan probe	AATGGTGGACCGCAACAACGCAATCT
IGF-I	Forward primer	GAGGAAGTGCAGGAAACAAGACC
	Reverse primer	ATTTGGCAGGTGTTCCGATG
	TaqMan probe	AACAGAAAATGCCACGTCACCGCA
Msx2	Forward primer	TCAGTCTGCCCTTCCCTATCAA
	Reverse primer	ATGGGAAGCACAGGTCTATGGA
	TaqMan probe	TTGCAAGCGGCATCCATATACAGCG
Dlx5	Forward primer	TCATGGCTACTGCTCTCCTACCT
	Reverse primer	CGTTCACGCTGTGATACTGGTAC
	TaqMan probe	TTCTTACCGCAAAGCGCTCAACCCAT
Cbfa1	Forward primer	CCTCACAAACAACCACAGAACC
	Reverse primer	AACCATTTAAACACCAGGGCC
	TaqMan probe	AAGTGCGGTGCAAACTTTCTCCAGGA

ALP = alkaline phosphatase; TGF-β_2 = transforming growth factor-β_2; IGF-I = insulin-like growth factor-I.

Table 2
Analysis of the effects of TAK-778 on the expression of alkaline phosphatase (ALP), osteocalcin, and type I collagen mRNA in rat bone marrow stromal cell cultures

	Time	ALP/GAPDH ($\times 10^{-2}$)		Osteocalcin/GAPDH ($\times 10^{-3}$)		Type I collagen/GAPDH	
		Control	TAK-778	Control	TAK-778	Control	TAK-778
Experiment 1	1 h	3.72 ± 0.31	3.54 ± 0.19	1.83 ± 0.14	1.80 ± 0.14	3.65 ± 0.37	3.81 ± 0.53
	4 h	3.54 ± 0.20	3.87 ± 0.21	1.51 ± 0.12	1.42 ± 0.13	4.00 ± 0.34	4.90 ± 0.48
	8 h	3.62 ± 0.18	4.08 ± 0.18	1.30 ± 0.14	1.29 ± 0.11	8.04 ± 1.22	5.14 ± 0.78
	24 h	3.27 ± 0.17	4.84 ± 0.16[a]	0.71 ± 0.04	0.96 ± 0.07	4.37 ± 0.18	3.70 ± 0.26
	48 h	5.73 ± 0.39	12.36 ± 0.54[a]	1.48 ± 0.12	6.06 ± 0.25[a]	5.58 ± 0.19	5.04 ± 0.68
Experiment 2	7 days	5.19 ± 0.25	33.76 ± 0.68[a]	6.55 ± 0.68	113.66 ± 4.94[a]	9.33 ± 0.82	10.75 ± 1.11

The cells were treated with TAK-778 (10^{-5} M) or vehicle (control) for the period indicated and total cellular RNA was prepared for analysis by quantitative RT-PCR using a PRISM7700 sequence detection system. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$).

[a] $P < 0.01$ vs. control, Student's *t*-test: in Experiment 1, Holm's correction for multiple comparisons was used.

β-glycerophosphate, 10^{-7} M dexamethasone, and antibiotics (80 μg/ml of gentamicin and 100 μg/ml of kanamycin). The cells were plated in 100-mm culture dishes (Falcon 3003, Becton Dickinson, Lincoln Park, NJ) containing 10 ml of the culture medium. The medium was changed every other day, and non-adherent blood cells were removed by washing the cells with the culture medium at each change. After 1 week, confluent cells in primary culture were harvested after treatment with 0.25% trypsin in calcium and magnesium-free phosphate-buffered saline (PBS) containing 0.2% EDTA (trypsin solution) and subcultured in 35-mm culture dishes (Falcon 3046, Becton Dickinson) at a cell density of 4×10^4 or 2×10^5 cells/well (day 0). All cell cultures used in this study were maintained at 37 °C in a humidified atmosphere of 5% CO_2–95% air.

TAK-778 was dissolved in a solution of *N*,*N*-dimethylformamide (DMF) at a concentration of 10 mM before use, diluted with culture medium to the designated concentrations, and added to subconfluent cultures from day 1 to the end of experiments. TAK-778 was freshly dissolved in DMF and diluted with culture medium at each medium change. Control cultures contained only the solvent. Culture medium was changed every other day.

2.3. RNA isolation and cDNA synthesis

Cells were washed with PBS, and total cellular RNA was isolated using STAT-60 (RNA/DNA/PROTEIN ISOLATION REAGENT, BIOTECX, Friendswood, TX). After treatment with RNase-free DNase (Promega, Madison, WI) to remove any DNA contamination, cDNA was prepared from total RNA (1000 ng) by reverse transcription (RT) with the following buffer and conditions: RT buffer (GIBCO BRL, Grand Island, NY) containing 50 mM Tris–Cl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 10 mM dithiothreitol, 0.5 mM each of dATP, dCTP, dGTP, and dTTP, 10 mg/ml of Random Primers (GIBCO BRL), and 200 units (U) of M-MLV reverse transcriptase (GIBCO BRL) in a final volume of 50 μl, with incubation for 1 h at 37 °C.

2.4. Real-time quantitative PCR

A real-time quantitative PCR method was used to detect accurately the change in osteoblast growth- and/or differentiation-related genes. The mRNAs of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, PE Applied Biosystems), alkaline phosphatase, osteocalcin, type I collagen, transforming growth factor (TGF)-β_2, insulin-like growth factor (IGF)-I, Msx2, Dlx5, and Cbfa1 were amplified, detected and quantitated in real time using the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA). The specific primers and the TaqMan probes were designed using Primer Express computer software (PE Applied Biosystems) (Table 1). The PCR mixture (25 μl total volume) consisted of TaqMan Universal PCR Master Mix containing AmpliTaq Gold, AmpErase UNG, dATP, dCTP, dGTP, and dUTP, primers (300 nM each), 200 nM TaqMan probe, and cDNA sample (10 ng). The cycling conditions included an initial phase of 2 min at 50 °C, followed by 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C.

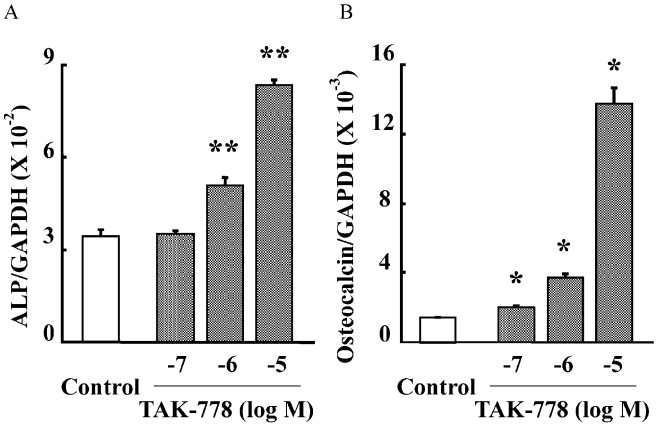

Fig. 2. Dose-dependent effects of TAK-778 on the expression alkaline phosphatase (A) and Osteocalcin (B) mRNA in rat bone marrow stromal cell cultures. The cells were treated with various concentrations of TAK-778 or vehicle (control) for 48 h. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$). * $P < 0.05$ and ** $P < 0.01$ vs. control, Dunnett's multiple comparison.

Table 3
Analysis of the effects of TAK-778 on the expression of TGF-β_2 and IGF-I mRNA in rat bone marrow stromal cell cultures

	Time	TGF-β_2/GAPDH ($\times 10^{-2}$)		IGF-I/GAPDH ($\times 10^{-2}$)	
		Control	TAK-778	Control	TAK-778
Experiment 1	1 h	2.74 ± 0.12	2.22 ± 0.23	1.53 ± 0.08	1.44 ± 0.05
	4 h	3.23 ± 0.20	3.99 ± 0.27	1.16 ± 0.09	1.48 ± 0.07
	8 h	4.12 ± 0.45	3.67 ± 0.39	1.31 ± 0.15	1.35 ± 0.07
	24 h	1.51 ± 0.05	2.04 ± 0.15	0.65 ± 0.07	1.26 ± 0.15
	48 h	2.35 ± 0.07	2.74 ± 0.24	1.48 ± 0.12	1.79 ± 0.24
Experiment 2	7 days	1.11 ± 0.07	2.63 ± 0.17[a]	7.00 ± 0.45	19.58 ± 1.29[a]

The cells were treated with TAK-778 (10^{-5} M) or vehicle (control) for the period indicated and total cellular RNA was prepared for analysis by quantitative RT-PCR using a PRISM7700 sequence detection system. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$).

[a] $P < 0.01$ vs. control, Student's *t*-test: in Experiment 1, Holm's correction for multiple comparisons was used.

2.5. Standard preparation

The cDNA was amplified using a Perkin-Elmer Cetus thermal cycler (Model 9600) with the PCR buffer (20 μl reaction solution) containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl, 0.4 mM each of specific primers, 1 U of AmpTaq DNA polymerase (PE Applied Biosystems), and 0.2 mM each of dNTPs (GIBCO BRL). The PCR conditions were 40 cycles of denaturation at 94 °C for 45 s, annealing for 45 s, and extension at 72 °C for 1 min. The PCR products were subjected to electrophoresis in 3% agarose gels, and stained with ethidium bromide. After the staining with ethidium bromide, the products as standard were purified (QUAEX Gel Extraction Kit; Qiagen, Hilden, Germany) and measured in a photometer, and molecular concentrations were calculated.

2.6. Assay quantification

Standard dilutions were made to produce a calibration standard curve covering a concentration of range 5×10^2–5×10^7 copies per well. Real-time quantitative PCR was then performed on these samples as described above. PCR standard curves were created using the threshold cycle defined as the point at which each reaction reaches the logarithmic portion of the PCR curve. The cycle number at which a reaction reaches this point in the curve is directly proportional to the amount of cDNA template in the PCR. Thus, this relationship allows for the creation of linear standard curves over a concentration range.

2.7. Transfection of Msx2 antisense

Transfections were performed with a specific delivery reagent, Ethoxylated Polyetylenimine (Gene Tools, LLC, Corvallis, USA). Antisense or sense oligonucleotides were added to the serum-free culture medium at a concentration of 1.4 mM with ethoxylated polyetylenimine. Three hours after transfection, the medium was replaced with regular growth medium containing TAK-778 and the cells were incubated for 48 h. Msx2 antisense or sense oligonucleotides were synthesized with the following sequences: Antisense: 5′TCC TAA GGG ACA GAG AGG GAA ATC A-3′: Sense: 5′-TCT TAA GAG ACA GAA AGT GAA ATC A-3′. In osteogenic cells transfected with Msx2 antisense oligonucleotide or with control oligonucleotide, either the antisense or sense oligonucleotide was nontoxic (data not shown).

2.8. Statistical analysis

All data are expressed as the mean ± S.E. Statistical differences were determined using Student's *t*-test or Holm's correction for multiple comparisons. Difference were considered significant at $P < 0.05$.

3. Results

3.1. Effect on mRNA expression of alkaline phosphatase and other osteoblast products

To determine the mechanism underlying the stimulatory effect of TAK-778 on osteoblast differentiation, we carried out a quantitative measurement of the transcriptional alteration of markers related to the expression of the osteoblast phenotype in the rat bone marrow stromal cell culture. As shown in Table 2, the treatment with TAK-778 (10^{-5} M) increased the level of both alkaline phosphatase and osteocalcin mRNA. Twenty-four hours of exposure had a significant effect on alkaline phosphatase mRNA and the stimulatory effects continued for up to 7 days. The stim-

Table 4
Effect of TAK-778 on the expression of Cbfa1, Dlx5, and Msx2 in rat bone marrow stromal cell cultures

	Messenger RNA/GAPDH		
	Cbfa1 ($\times 10^{-3}$)	Dlx5 ($\times 10^{-2}$)	Msx2 ($\times 10^{-4}$)
Control	5.54 ± 0.71	1.03 ± 0.27	2.86 ± 0.34
TAK-778	5.02 ± 0.75	1.48 ± 0.28	5.68 ± 0.68[a]

The cells were treated with TAK-778 (10^{-5} M) or vehicle (control) for 4 h. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$).

[a] $P < 0.01$ vs. control, Student's *t*-test.

Table 5
Analysis of the effects of TAK-778 on the expression of Msx2 mRNA in rat bone marrow stromal cell cultures

	Time	Msx2/GAPDH ($\times 10^{-4}$)	
		Control	TAK-778
Experiment 1	1 h	1.33 ± 0.27	1.22 ± 0.16
	4 h	1.05 ± 0.10	1.95 ± 0.19[a]
	8 h	1.35 ± 0.16	4.71 ± 1.55
	24 h	1.22 ± 0.12	3.65 ± 0.13[a]
	48 h	1.24 ± 0.21	3.63 ± 0.40[a]
Experiment 2	7 days	2.01 ± 0.21	6.83 ± 0.40[a]

The cells were treated with TAK-778 (10^{-5} M) or vehicle (control) for the period indicated and total cellular RNA was prepared for analysis by quantitative RT-PCR using a PRISM7700 sequence detection system. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$).

[a] $P < 0.01$ vs. control, Student's *t*-test: in Experiment 1, Holm's correction for multiple comparisons was used.

ulatory effects of TAK-778 were dose-dependent (Fig. 2). These results indicate that TAK-778 regulates the expression of alkaline phosphatase and osteocalcin at the transcriptional level. On the other hand, TAK-778 did not affect the level of type I collagen mRNA, suggesting that TAK-778 enhances collagen synthesis through post-transcriptional mechanisms (Table 2). Continuous treatment with TAK-778 for 7 days resulted in increases in the expression of both TGF-β and IGF-I mRNA, while an initial 48-h exposure had no effect on these indices (Table 3). These results reveal that these autocrine/paracrine growth factors are not the initial targets of TAK-778 during osteoblast differentiation. TAK-778 had no effect on the levels of BMP-2, 4, BMPR-IA, and BMPR-II mRNA during this culture period (data not shown).

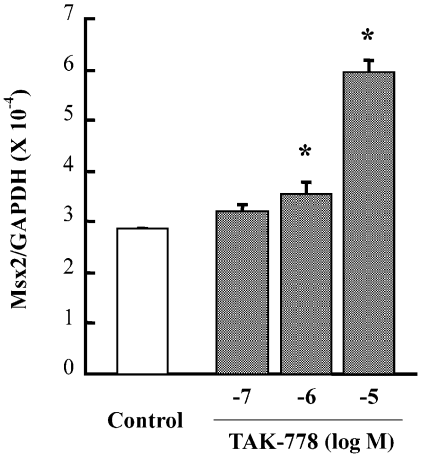

Fig. 3. Dose-dependent effects of TAK-778 on Msx2 mRNA in rat bone marrow stromal cell cultures. The cells were treated with or without various concentrations of TAK-778 for 48 h. Total cellular RNA was prepared for analysis by quantitative RT-PCR using a PRISM7700 sequence detection system. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$). * $P < 0.05$ vs. control, Dunnett's multiple comparison.

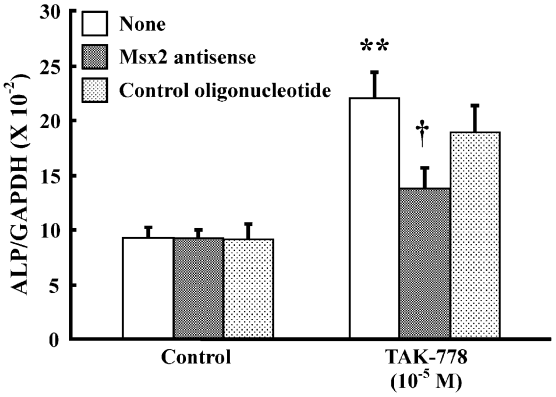

Fig. 4. Effect of Msx2 antisense and control oligonucleotides on TAK-778-induced alkaline phosphatase mRNA expression in rat bone marrow stromal cell cultures. The cells were treated with Msx2 antisense and control oligonucleotides for 3 h and then treated with or without TAK-778 (10^{-5} M) for 48 h. Total cellular RNA was prepared for analysis by quantitative RT-PCR using a PRISM7700 sequence detection system. Data were normalized to GAPDH mRNA. Values are the mean ± S.E. ($n = 5$). ** $P < 0.01$ vs. control (none), Student's *t*-test. † $P < 0.05$ vs. TAK-778 (none), Tukey test.

3.2. Effect on mRNA expression of transcription factors involved in osteoblast differentiation

We assessed the effect of TAK-778 on the mRNA expression of Msx2, Cbfa1, and Dlx5 in a rat bone marrow stromal cell culture. As shown in Table 4, TAK-778 significantly stimulated the mRNA expression of Msx2, but not Cbfa1 or Dlx5. An initial 4-h exposure was long enough to upregulate the expression. The stimulatory effect was dose-dependent and continued for 7 days (Table 5 and Fig. 3). These results indicated that the expression of Msx2 seems to be one of initial actions induced by TAK-778.

3.3. Effect of Msx2 antisense oligonucleotide

To elucidate the relationship between TAK-778-induced osteoblast differentiation and the enhancement of Msx2 mRNA expression, we transfected rat bone marrow stromal cells with Msx2-antisense oligonucleotide. The transfection with the antisense oligonucleotide resulted in a significant decrease in the TAK-778-stimulated expression of alkaline phosphatase mRNA (Fig. 4). Neither antisense nor control oligonucleotide had an effect on the basal level of alkaline phosphatase mRNA. The findings suggest that the expression of Msx2 is required for the stimulatory effects of TAK-778 on alkaline phosphatase mRNA expression.

4. Discussion

We previously reported that TAK-778, a novel chemical compound, enhances the expression of the osteoblast phenotype, that is, alkaline phosphatase activity, osteocalcin

secretion, TGF-β and IGF-I production (Notoya et al., 1999). In the present study, we demonstrated that TAK-778 stimulated the expression of these proteins at the transcriptional level in the same culture. We also showed that these TAK-778-induced actions occurred sequentially. Twenty-four hours of exposure to TAK-778 had significant effects on the expression of alkaline phosphatase and osteocalcin mRNA, while an initial 48-h exposure had no effect on TGF-β_2 and IGF-I mRNA, suggesting that these autocrine/paracrine growth factors are not the initial targets of TAK-778 in osteoblast differentiation.

An initial 4-h exposure to TAK-778 upregulated the expression of Msx2 mRNA. Msx2 is part of the *Drosophila msh* gene (Bell et al., 1993) and expressed in numerous tissues at many stages of development (Davidson, 1995). There is a growing evidence of the important role for Msx2 in osteoblast growth and differentiation. MSX2 is expressed and regulated by 1,25-dihydroxy vitamin D3 in human bone-derived cells (Hodgkinson et al., 1993). Msx2 inhibits the osteocalcin promoter in transfected MC3T3-E1 cells, but stimulates the osteocalcin promoter in ROS 17/2.8 cells (Towler et al., 1994). Overexpression of Msx2 driven by the mouse Msx2 promoter caused an increase in the number of proliferative osteoblastic cells in the osteogenic fronts of calvarial bones in transgenic mice (Liu et al., 1999). In more recent studies, it was shown that a deficiency of Msx2 in mice caused defects in bone growth, resulting from both a defective proliferation of osteoblast progenitors and reduced expression of the osteoblast phenotype (Satokata et al., 2000). These findings and our results raise the possibility that the Msx2 gene is strongly involved in TAK-778-stimulated osteogenesis. In fact, transfection of Msx2 antisense oligonucleotide blocked TAK-778-induced alkaline phosphatase mRNA expression while transfection of control oligonucleotide had no effect, indicating that TAK-778 may stimulate alkaline phosphatase activity through the expression of Msx2 in rat bone marrow stromal cells. Upstream signaling of the Msx2 expression remains unclear. To clarify this, a gene array analysis of the cells following treatment with TAK-778 is now under way.

There are several similarities and differences between the pharmacological profiles of TAK-778 and those of other endogenous osteogenic agents, such as BMPs and FGFs. These agents stimulate cellular alkaline phosphatase activity and bone-like nodule formation in rat bone marrow stromal cell cultures (Hanada et al., 1997). Also, both BMPs and FGFs induce MSX expression (Hollnagel et al., 1999; Kim et al., 1998). On the other hand, BMPs, unlike TAK-778, have been shown to induce the expression of Cbfa1, a prerequisite transcription factor for osteoblast differentiation, in experiments performed in knockout mice (Ducy et al., 1997; Komori et al., 1997; Gori et al., 1999). Actually, BMPs induce the development and expression of osteogenic lineages in uncommitted mesenchymal C3H10T1/2 cell cultures (Katagiri et al., 1990; Wang et al., 1993), whereas TAK-778 does not (Notoya et al., 1999). This may lead to the in vivo phenomenon whereby only BMPs induce ectopic bone formation. Basic FGF has been reported to suppress both the expression of Cbfa1 mRNA (Tsuji and Noda, 2001) and the features of differentiated osteoblasts (Rodan et al., 1989; Hurley et al., 1993). This effect, which TAK-778 does not have, may be due to the powerful growth-promoting properties of bFGF (Gospodarowicz et al., 1987; Gospodarowicz, 1990), since the osteoblast phenotype is heavily influenced by proliferative status (Owen et al., 1990).

TAK-778 had no effect on the mRNA level of type I collagen during the culture period although this compound increased the protein level of soluble collagen released into the culture medium (Notoya et al., 1999). In general, there is a series of evidence showing that regulation occurs via multiple steps resulting in an increase in collagen production, including transcriptional and posttranscriptional mechanisms (Tajima et al., 1995; Kay et al., 1996). TAK-778 may stimulate the synthesis of type I collagen by altering the rate or efficacy of posttranscriptional steps.

In conclusion, our findings indicate that TAK-778 stimulates the expression of osteoblast differentiation-related proteins at the transcriptional level in osteogenic cells and also suggest that Msx2 is required for TAK-778-induced expression of alkaline phosphatase, probably resulting in the enhancement of osteoblast differentiation. Thus, TAK-778 may be a useful pharmacological tool that induces Msx2 expression in osteoblast-lineage cells and the study of TAK-778 may provide information about the mechanisms of osteoblast differentiation as well as osteogenesis.

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